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<p>We have proposed a novel approach to the cloning of tumor suppressor genes in prostate cancer. We have developed methods for the transfer of large pieces of human DNA cloned into bacterial artificial chromosomes (BACs) into human prostate cancer cell lines. For this purpose, we target the BACs with drug resistance and other markers that will allow them to be stably transferred into the cell lines, and then transfer them into several different prostate cancer cell lines. For the next stage of this project, we plan to use several different assays to test the ability of the transferred human DNA to revert (render less tumorigenic) the neoplastic phenotype of the cancer cell lines. These assays will include morphological changes in the cells, doubling time, growth in soft agar, and tumorigenicity in immunodeficient mice. The assays will be used to determine if a particular BAC contains a gene that reverts the transformed phenotype of the cell line, and therefore contains a human tumor suppressor gene. BACs that revert cell lines in this assay will then be fragmented or subdivided, and the subclones tested in our assays. In this way, the tumor suppressor gene on the BAC will be localized precisely.</p>			
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FOREWORD

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INTRODUCTION

Cloning and study of tumor suppressor genes in prostate cancer has the potential to expand our knowledge of the pathogenesis of this disease and improve diagnosis, risk stratification, and therapy. We have proposed a novel approach to the cloning of tumor suppressor genes in prostate cancer. We have developed methods for the transfer of large pieces of human DNA cloned into bacterial artificial chromosomes (BACs) into human prostate cancer cell lines. For this purpose, we target the BACs with drug resistance and other markers that will allow them to be stably transferred into the cell lines, and then transfer them into several different prostate cancer cell lines. For the next stage of this project, we plan to use several different assays to test the ability of the transferred human DNA to revert (render less tumorigenic) the neoplastic phenotype of the cancer cell lines. These assays will include morphological changes in the cells, doubling time, growth in soft agar, and tumorigenicity in immunodeficient mice. The assays will be used to determine if a particular BAC contains a gene that reverts the transformed phenotype of the cell line, and therefore contains a human tumor suppressor gene. BACs that revert cell lines in this assay will then be fragmented or subdivided, and the subclones tested in our assays. In this way, the tumor suppressor gene on the BAC will be localized precisely. The fragments of the BAC will then be used to obtain the cDNA for the gene. Initially, we will concentrate our efforts to clone a tumor suppressor gene on human chromosome 17q. However, our approach is potentially applicable to the cloning of any human prostate tumor suppressor gene, and thus will be of major importance.

BODY: RESEARCH ACCOMPLISHMENTS ASSOCIATED WITH EACH TASK OUTLINED IN THE STATEMENT OF WORK

The Technical Objectives/Specific Aims of the Project were as follows:

- (1) To develop methods for the efficient and stable transfer of human DNA segments cloned in yeast artificial chromosomes (YACs) or BACs into human prostate cancer cell lines.
- (2) To localize the human tumor suppressor gene located on a contig of YACs or BACs on chromosome 17q that produces phenotypic reversion of human prostate cancer cell line(s).

The Approved Statement of Work was as Follows:

Task 1: To develop methods for the efficient and stable transfer of human DNA segments cloned in YACs or BACs into human prostate cancer cell lines (Months 1-18).

- Target YACs with the neomycin-resistance vector and selected YACs with the GFP cassette (Months 1-6).
- Test for the ability to introduce the YACs into prostate cancer cell lines using polyethylene glycol mediated fusion (Months 3-9).
- Test for the ability to introduce the YACs into prostate cancer cell lines using lipid-mediated transfection (Months 3-9).
- Test for the ability to introduce the YACs into prostate cancer cell lines using microinjection (Months 6-18).

Task 2: To isolate the human tumor suppressor gene located on a contig of YACs or BACs on chromosome 17q that produces phenotypic reversion of human prostate cancer cell lines (Months 12-30).

- Transfer BACs into the cell lines by co-transfection, and obtain neomycin-resistant clones (Months 12-18). (Alternatively, use one of the methods outlined in Task 1, above, to transfer YAC DNA into the cell lines).
- Assay the clones for the presence of YAC or BAC DNA in the clones by PCR for the vector "arms," or by PCR for polymorphisms present on the YAC or BAC (Months 12-18).
- Test neomycin-resistant clones for cloning efficiency in soft agar and assess doubling time, and record morphology (Months 16-30).
- Inject promising clones into immunodeficient mice, and observe for tumor formation (Months 18-30). Approximately 60 animals will be needed.
- Fragment YACs or BACs, transfer them into cell lines, and assay for reversion (to determine the exact location of the gene on the YACs or BACs; [Months 18-30, depending on how quickly a reverting clone is obtained in the previous steps]).

Obtaining and studying cDNAs corresponding to the gene will probably be outside the time frame of the New Investigator Proposal, but would be an initial objective for Phase II.

Progress in the first year of the project:

a) Target YACs with the neomycin-resistance vector and selected YACs with the GFP cassette (Months 1-6): We had successfully targeted YACs with a neomycin-resistance vector prior to the submission of the initial grant proposal, using methods published previously by others (1; 2). These data were summarized in the original grant proposal.

After the submission of the grant proposal, it became apparent that the general progress in the human genome project (see ref. (3) for an example) required a re-assessment of our strategy of using YACs. It has become clear that YACs have a very high frequency of chimerism. This was realized only when a substantial number of well-mapped genetic markers became available. The majority of YACs that have been examined with a reasonable number of markers have been found to contain several pieces of DNA that are non-contiguous in the actual genome. This has created problems for mapping and sequencing efforts, and it is noteworthy that all the major genome sequencing projects have abandoned YACs in favor of BACs (or even smaller clones, such as PACs (P1 artificial chromosomes)). It is particularly a problem when we wish to assay genes on a YAC for potential functional properties (e.g., in our reversion assay). In light of these new developments, we have abandoned working with YACs in all phases of this study.

b) Test for the ability to introduce the YACs into prostate cancer cell lines using polyethylene glycol mediated fusion (Months 3-9): For the reasons described above (section a), these studies have been abandoned in favor of analogous experiments with BACs (see section e).

c) Test for the ability to introduce the YACs into prostate cancer cell lines using lipid-mediated transfection (Months 3-9): For the reasons described above (section a), these studies have been abandoned in favor of analogous experiments with BACs (see section e).

d) Test for the ability to introduce the YACs into prostate cancer cell lines using microinjection (Months 6-18): For the reasons described above (section a), these studies have been abandoned in favor of analogous experiments with YACs (see section e).

(e) Transfer of BACs: Bacterial artificial chromosomes (BACs) are large circular molecules of DNA (average insert size, 200 kb) that propagate autonomously in E. coli (4). Since the submission of the original grant proposal, several groups have now reported methods for the targeting of BACs with a drug resistance marker that would allow for their efficient transfer into mammalian cells (5; 6). Previously, another group had been successful in introducing functional BAC DNA into mouse embryos by microinjection, without the use of a selectable marker (7). In the original grant proposal, we provided data on the successful transfer of mouse BACs into human prostate cancer cell lines using co-transfection (8) with a selectable marker. We tested for the presence of BAC DNA in the cell lines by hybridization, and found that a sizable proportion, possibly all, of the BAC DNA had been successfully transferred into the human prostate cancer cell line. We anticipate that identical methods can be used with human BACs.

(f) Obtaining BACs from the region of interest: The development of BAC contigs for various regions of the human genome has lagged behind the work done with YACs. When we wrote the original grant proposal, we stated that "we expect that work with BACs will soon catch up with the enormous progress made to date with YACs, in view of the great interest in using BACs in the sequencing phase of the human genome project (3). Therefore, we expect to obtain BACs covering 17q within the next year." It is clear that, in retrospect, we were overly optimistic about the time in which 17q BACs would become available. There has been remarkable progress in obtaining BACs for many regions of the human genome since the submission of this proposal, but this progress has been rather uneven. Virtually complete BAC contigs (>90% coverage) for human chromosome 22 are available, and several other chromosomes have extensive coverage (see, for example, the maps from the Sanger Center at www.sanger.ac.uk). However, progress for chromosome 17 has been slower. We believe that the most likely source of BACs for our project will come from the genome-wide sequencing effort of Craig Venter's group at Celera Genomics. One advantage of the approach taken by this group is that all regions of the human genome will be mapped simultaneously. Although the sequence generated by the Celera group will probably contain numerous small gaps, they anticipate that it will be sufficient to map the vast majority of the BACs for which end-sequencing data is available (3). Therefore, we remain highly confident that BACs will become available for the 17q region in a reasonable period.

KEY RESEARCH ACCOMPLISHMENTS

None as yet.

REPORTABLE OUTCOMES

None as yet.

CONCLUSIONS

This proposal was designed to both test methodologies (i.e., the transfer of BACs and/or YACs to prostate cancer cell lines), and also to isolate genes (i.e., a putative prostate cancer tumor suppressor genes located on 17q). Our studies have demonstrated that our original hypothesis was correct (i.e., BAC DNA could be transferred into human prostate cancer cell lines at reasonable efficiency). The major obstacle to future work is basically beyond our control: we require a BAC contig spanning the region of interest, and this BAC contig can best be obtained as part of the human genome project. As the human genome project is proceeding ahead of schedule, we anticipate that a BAC contig will become available soon. For this reason, we remain optimistic that the original objectives of the proposal can be met.

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